15. Microbial Exchange

Experiment AR-002*

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ABSTRACT

The purpose of the Microbial Exchange Experiment was to evaluate components of the infectious disease process in space flight by measuring alterations in three factors: (1) the composition of the microbial populations inhabiting the crewmembers and the spacecraft, (2) the ability of each crewmember's defense mechanism to resist infection, and (3) the ability of certain microorganisms to originate infections. The impetus for performing the experiment arose from the variety of in-flight and ground-based microbial studies in the United States and the U.S.S.R., the results of which have suggested that the conditions of space flight may alter man and microorganisms in such a way that the normal fine balance between them may be adversely affected. The monitoring of two separate crews, which differed microbiologically and immunologically, provided an opportunity to study in-flight cross-contamination patterns. Because the Apollo-Soyuz Test Project crewmembers came from widely different geographical and ecological areas, it was possible to identify specific, naturally occurring, marker microorganisms for detailed analysis.

This report evaluates the operational aspects associated with the experiment, the activities of medically important microorganisms recovered from the six Apollo crewmembers (three prime and three backup) and four Soyuz crewmembers (two prime and two backup), and the activities of the major groups of microorganisms recovered from the 10 sample sites of each crewmember. Most activities were performed as planned, and a large percentage of the anticipated data was obtained. A variety of potential pathogens was recovered from each of the prime and backup crewmembers before and after flight. However, no disease events were reported. *Candida albicans* and *Staphylococcus aureus* (type 52, 52A, 80, 81) were shown to be transferred from one crewmember to another during the flight. No other medically significant changes in the microbial population were observed.

Within the microbial population of the 10 sampled crewmembers, there was no significant difference between the total load of aerobic bacteria inhabiting the integumentary surface of each, or between the total microbial load in the upper respiratory tract of each. The total load recovered from each crewmember on the day of return from space flight was not significantly different from the preflight mean, contraindicating discernible

*The Microbial Exchange Experiment is one of five experiments that incorporated joint activities of the U.S. and U.S.S.R. crews. The joint experiment designation was "Experiment AS-2."

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flight-affected changes in the size of the population of aerobic bacteria. The theories that proposed "simplification" of the microbial population, "dysbacteriotic" changes in the oral autoflora, and alteration in the immune potential were evaluated. No data were obtained to corroborate these hypotheses.

INTRODUCTION

The problems concerning the occurrence of microbial contamination and infectious diseases during space flight have been evaluated repeatedly following U.S. and U.S.S.R. manned missions. Scientists in the U.S.S.R. have been concerned with crew microbiological profiles and natural bactericidal activity since their first manned mission. Twelve years ago, Alekseyeva (ref. 15-1) reported that the composition and properties of the autoflora of cosmonauts remained within normal limits during preflight evaluations but that there were occasional postflight changes. An increase in oral autoflora was accompanied by dysbacteriosis in which the normal throat flora were almost entirely crowded out by Gram-negative, nonhemolytic diplococci. It was also reported that an increase in the number of microbes in the deep layers of the skin was caused by the appearance of masses of hemolytic micrococci (ref. 15-1). More recently, Nefedov et al. (ref. 15-2) have reported that alterations in microbial flora have been observed on several flights and appear to be dependent on "environmental parameters," the duration of the exposure to different environmental factors, and the work-rest schedules. The same authors point out that one of the most consistent phenomena appears to be the interchange of microorganisms between subjects (ref. 15-2). The advent of the U.S.S.R. space station, Salyut, enabled in-flight samples to be taken from the three crewmembers. Postflight analyses of these samples revealed changes in autoflora and microbial interchange.[1] However, the death of the crewmembers prevented postflight sampling and analysis.

The interest of U.S.S.R. investigations in autoflora has not been restricted to cosmonauts. Alekseyeva and Volkova (ref. 15-3) have reported studies with "space dogs" (dogs that have experienced space flight) since 1960. They reported that whereas acceleration and vibration on the ground had no effect on the microflora, the space dogs had intestinal bacilli in their mouths after 2 days of space flight. The authors interpreted the presence of intestinal bacilli to be a result of a space-flight-mediated decrease in the immunological activity of the subjects.

The interest of the U.S.S.R. space agency concerning this research, and the philosophy that led to participation in the Microbial Exchange Experiment, was offered during the first meeting of the U.S.-U.S.S.R. Joint Working Group in Space Biology and Medicine in Moscow.[2] During the meeting, U.S.S.R. investigators made the following observation.

> The results of the study of the automicroflora of the integumentary tissues of the cosmonauts, indicating the development of dysbacteriotic changes in its composition during the space flight, have necessitated more detailed scientific studies in this area. Studies to clarify the role of the environment of objects in space in terms of the appearance of unfavorable changes in the composition of the automicroflora of the cosmonauts are most important in this respect.

Corresponding NASA studies began with comparisons between preflight and postflight specimens collected during one of the Gemini flights (ref. 15-4). In this first U.S. microbial analysis, the investigators reported a decrease in the number of types and an increase in the total number of microorganisms recovered after the flight. Also, there was evidence of microbial transfer between

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[1]L. I. Kakurin, data presented at the second meeting of the U.S.-U.S.S.R. Joint Working Group in Space Biology and Medicine, NASA Lyndon B. Johnson Space Center, May 1972.

[2]Data presented at the first meeting of the U.S.-U.S.S.R. Joint Working Group in Space Biology and Medicine, Moscow, 1971.

the crewmembers. When evaluating these findings in reference to the total clinical picture, Berry (ref. 15-5) indicated that the observed microbial changes may not be compatible with the health and welfare of the crewmembers. In a survey of 26 chamber tests conducted by the National Academy of Sciences between 1962 and 1970, evidence was presented which indicates that microbial exchange is not infrequent and that it may contribute to medically significant events (ref. 15-6).

Detailed analyses of preflight and postflight samples from Apollo 14 crewmembers revealed (1) an intercrew transfer of *Staphylococcus aureus*, (2) an 830-percent increase in the quantitation of aerobic bacteria, and (3) a transfer of microorganisms from crewmembers to the command module hardware (ref. 15-7). A dramatic postflight reduction in the number of mycological species recovered from the Apollo 14 crewmembers was also observed with the Apollo 15 crew (ref. 15-8).

With the advent of the Skylab missions, it became possible to evaluate the microbial alterations resulting from space flights with durations as long as 85 days. Complete autoflora studies were conducted on each of the prime crews (refs. 15-9 and 15-10). Preliminary evaluations show that, although gross contamination of the Skylab environment did occur and although there were several in-flight disease events (presumably of microbial origin), such events did not prove to be limiting hazards for long-term space flight. Evaluation of the major groups of microorganisms, comprising the microbial population tested, tended to support the theory of microbial simplification for anaerobic bacteria (refs. 15-2, 15-11, and 15-12) but not for other microbes. Intercrew transfer of pathogens was indicated. The data did not support the theory of postflight microbial shock (refs. 15-12 to 15-14).

The review by Fox (ref. 15-15) indicates clearly that the results of the early NASA autoflora studies were analogous to those studies performed in the U.S.S.R. Both studies have indicated significant alterations in the microbial population as well as definite transfer of species between crewmembers.

The Microbial Exchange Experiment was designed to monitor quantitatively the microbial load of all crewmembers and of selected inner surfaces of both the Apollo and Soyuz spacecraft and to evaluate the status of certain components of the immune mechanism of each crewmember. To accomplish this objective, an evaluation of the normal autoflora and immunocompetence level of each crewmember was established before flight and through repeated sampling and analyses. Therefore, quantitative and qualitative changes occurring during or after flight could be measured and evaluated, and instances of intercrew transfer of marker organisms could be identified. At the same time, certain immunological parameters of the blood and saliva of each crewmember were studied to detect changes in the ability of the individual to resist infection.

METHODS AND MATERIALS

Baseline Microbial Specimen Collection

Specimens were to be collected from the 5 prime and 5 backup crewmembers and from 15 areas on the inner surfaces of each spacecraft at specific times before, during, and after the flight as given in table 15-I. During each preflight and postflight sampling period, 10 microbial specimens were collected from each crewmember (table 15-II). Calcium alginate swabs, wetted in 0.3-millimolar phosphate buffer, were used to sample each of the seven body surface areas. This swab technique was the only collection method compatible with the flight program. Although it is recognized that subsurface microbes may be overlooked by this method, procedures for sampling subsurface areas were not acceptable. Dry calcium alginate swabs were used to sample the surfaces of the tonsils and the posterior pharyngeal vault before collection of the gargle specimen. For the latter sample, the subject gargled with 0.3-millimolar phosphate buffer followed by a repeated rinse of the teeth with the same solution. Swabs were placed in 5 cm^3 of 0.3-millimolar phosphate buffer for transport to the laboratory. Analysis of all samples was initiated within 1 hour of specimen collection.

TABLE 15-I.—*Sample Collection Schedule*

Sample	Day of collection											
	Days before flight (a)						During flight	Days after flight (a)				
	F−45	F−30	F−15	F−7	F−1	F−0		R+0	R+3	R+7	R+15	R+30
Blood		X	X		X		X	X[b]	X[b]			
Spacecraft microflora								X				
Saliva	X	X	X	X		X		X		X[b]	X[b]	X[b]
Crew microflora	X	X	X	X		X	X	X		X[b]	X[b]	X[b]

[a] F−0 = day of launch and R+0 = day of recovery.
[b] Prime crewmembers only.

TABLE 15-II.—*Sample Collection Sites*

Sample designation	Sample area
Hair	20-cm^2 area of hair (and scalp) on top of head
Ears	Right and left external auditory canals with two revolutions of each swab in each ear canal
Neck	20-cm^2 area below hairline at base of neck
Nares	Internal area of both nostrils
Throat swab	Surfaces of tonsils and posterior pharyngeal vault
Hands	20-cm^2 area on right and left palms
Axillae	20-cm^2 area below hair on each side
Groin	5-cm strip from rear to front on right and left inguinal area between legs
Toes	Area between the two smallest toes of each foot
Gargle	10 cm^3 of phosphate buffer used as gargle and washed through oral cavity three times

In-Flight Microbial Specimen Collection

In addition to the baseline samples previously described, in-flight samples were obtained from all five prime crewmembers and both spacecraft between 77:40 and 78:30 ground elapsed time (GET). A specially developed sample collection device was used for this set of samples (fig. 15-1). This device consisted of a cotton-tipped Teflon swab on a capillary tube that contained conservation fluid to keep the microorganisms alive. Each swab was housed within an airtight case to prevent desiccation. Groups of swabs were organized in Beta-cloth retaining bags (fig. 15-2). One bag of this type was provided for each of the following: (1) swabs used for Soyuz spacecraft samples, (2) swabs used for Apollo spacecraft samples, (3) swabs used for Apollo commander (ACDR) and Soyuz commander (SCDR) samples that were taken in the Soyuz, (4) swabs used for Soyuz flight engineer (SFE), docking module pilot (DMP), and command module pilot (CMP) samples that were taken in the Apollo spacecraft.

All four sets of swabs were launched in the Soyuz spacecraft. The two kits to be used in the Apollo were transferred from the Soyuz at the end of the first joint activity (58:20 GET) and

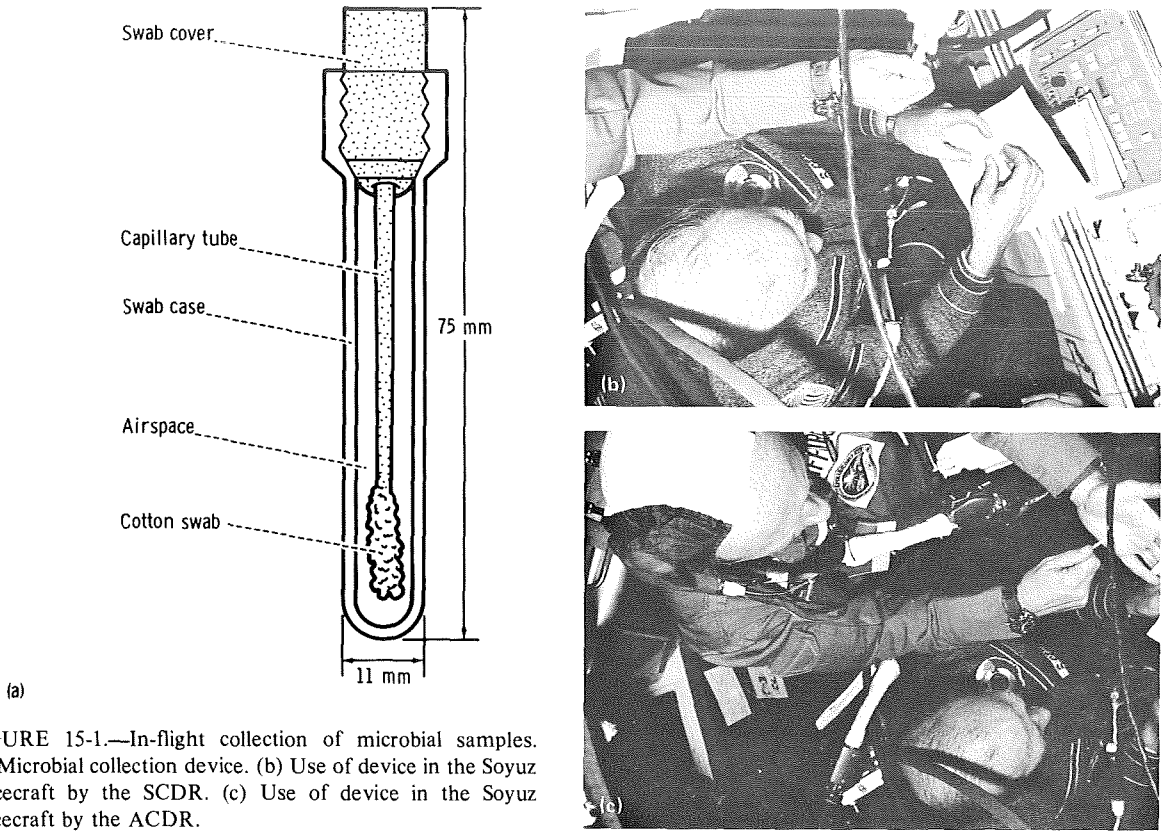


FIGURE 15-1.—In-flight collection of microbial samples. (a) Microbial collection device. (b) Use of device in the Soyuz spacecraft by the SCDR. (c) Use of device in the Soyuz spacecraft by the ACDR.

returned to the Soyuz near the end of the last joint activity (79:50 GET). All samples collected during flight were returned to Moscow for preliminary analysis and division between the U.S. and U.S.S.R. laboratories as outlined in the following discussion. In-flight samples consisted of the first six areas outlined in table 15-II as well as the spacecraft sites presented in table 15-III. It should be noted that Soyuz spacecraft samples C-7 through C-15 were taken from the part of the spacecraft not returned to Earth. Accordingly, these samples were not collected after the flight. To assist in spacecraft sample collection, black lines demarking a 100-cm^2 area had previously been painted at each of the specified sites as shown in the examples illustrated in figure 15-3.

Because the specimens collected during flight had to be returned to the laboratory for analysis, considerable delay was unavoidable. To equate results from these specimens with results from specimens collected before and after flight, a group of samples was collected the morning of launch and immediately upon return from the flight. These samples were held at room temperature for 4 days to simulate the delay imposed on the in-flight specimens. These comparative specimens were collected from all 10 crewmembers and both spacecraft using the same type swabs used during flight.

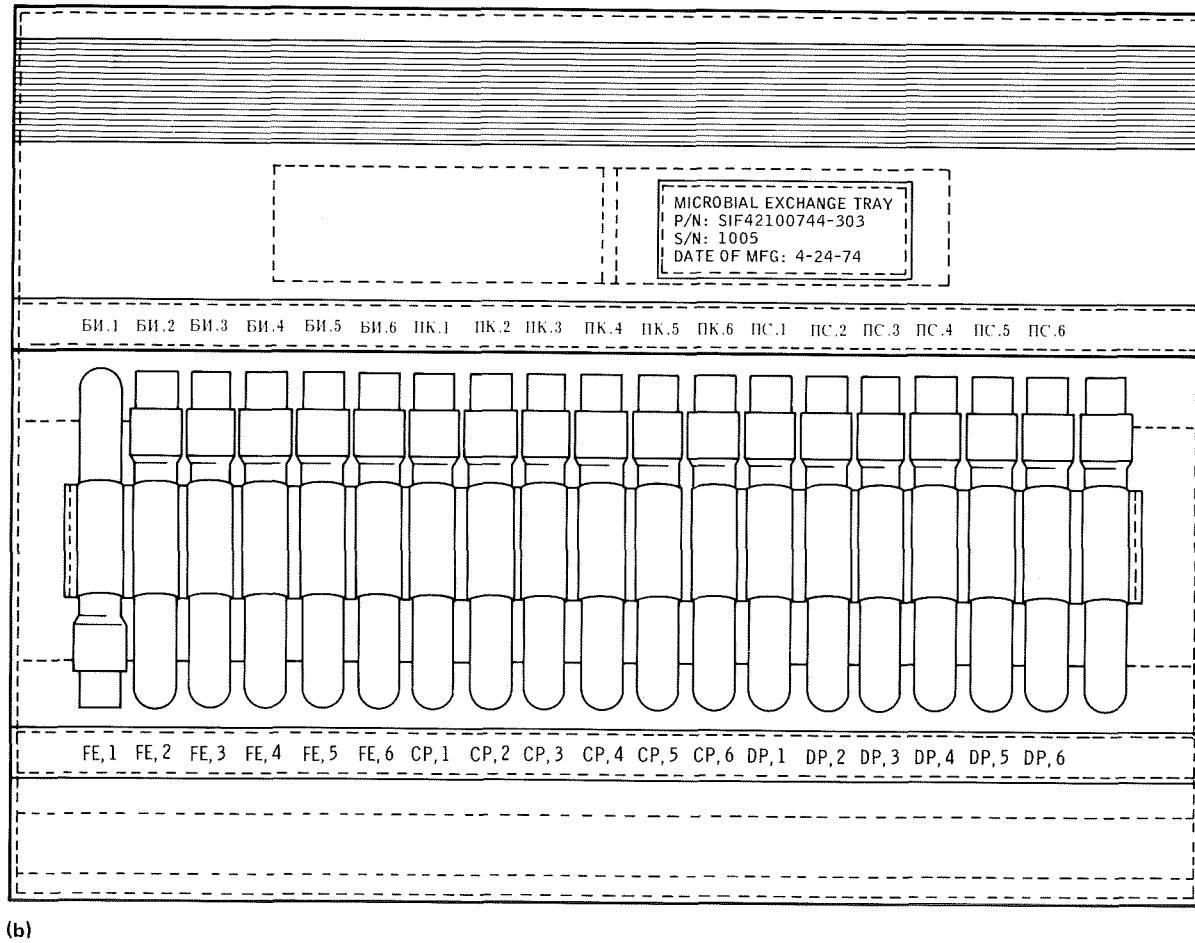
Collection of Salivary Parotid Fluid

Saliva from the parotid gland was collected from each of the 10 crewmembers before flight and from the 5 prime crewmembers after flight according to the schedule shown in table 15-I. The special saliva collection device, shown in figure 15-4, was attached by pressure to the inner surface of the oral cavity over the opening of the parotid duct. The parotid fluid was collected for 10 minutes with the flow being stimulated by a sour

candy drop placed in the subject's mouth. The resulting saliva was frozen at 233 K (−40° C) and stored for future analysis. No saliva was collected during the flight.

Blood Collection and Serum Preparation

Venous blood was collected from the antecubital fossae of each of the 10 participating crewmembers before flight and from the 5 prime crewmembers after flight as outlined in table 15-I. From each sample, whole blood was allowed to clot for serum separation. The resulting serum was used to determine humoral antibody levels and bactericidal activity.


MICROBIAL EXCHANGE TRAY
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FE,1 FE,2 FE,3 FE,4 FE,5 FE,6 CP,1 CP,2 CP,3 CP,4 CP,5 CP,6 DP,1 DP,2 DP,3 DP,4 DP,5 DP,6
(b)

FIGURE 15-2.—Beta-cloth sample collection kit used for crewmember samples in the Apollo spacecraft. (a) Closed kit. (b) Open kit showing sample collection devices in place.

TABLE 15-III.—Location of Sampling Sites on Inner Surfaces of Spacecraft

Sample no.	*Location in Soyuz*	*Sample no.*	*Location in Apollo*
C-1	SCDR couch seat	A-1	Left X-X headstrut
C-2	SFE couch seat	A-2	Right X-X footstrut
C-3	Control panel in the descent vehicle	A-3	Crew couch, right stabilizer beam
C-4	Surface of gas mixture regulator	A-4	Tunnel area
		A-5	Right floodlight
C-5	Surface near bulkhead hatch in descent vehicle	A-6	Left rotational hand controller pistol grip (both sides)
C-6	Descent vehicle hatch cover under the handwheel	A-7	Right girth shelf above panel 278
C-7	Flush side of sanitary device in the orbital module (OM)	A-8	Panel 325 below window
C-8	Top of food locker table in OM	A-9	Above left girth shelf (above U-3)
C-9	Couch seat in OM	A-10	Cover plate, ORDEAL stowage locker (U-3)
C-10	Surface of food locker in OM near panel	A-11	B-6 door behind waste stowage bag
C-11	Front surface of the couch backrest in the OM	A-12	Inside door of food locker (L-3)
C-12	Grid near fan in OM		
C-13	Floor surface near OM food locker	A-13	Top of video tape recorder module
C-14	Transfer hatch under the handwheel	A-14	Panel 251, waste management dump
C-15	Surface of transfer hatch	A-15	Forward of panel 225

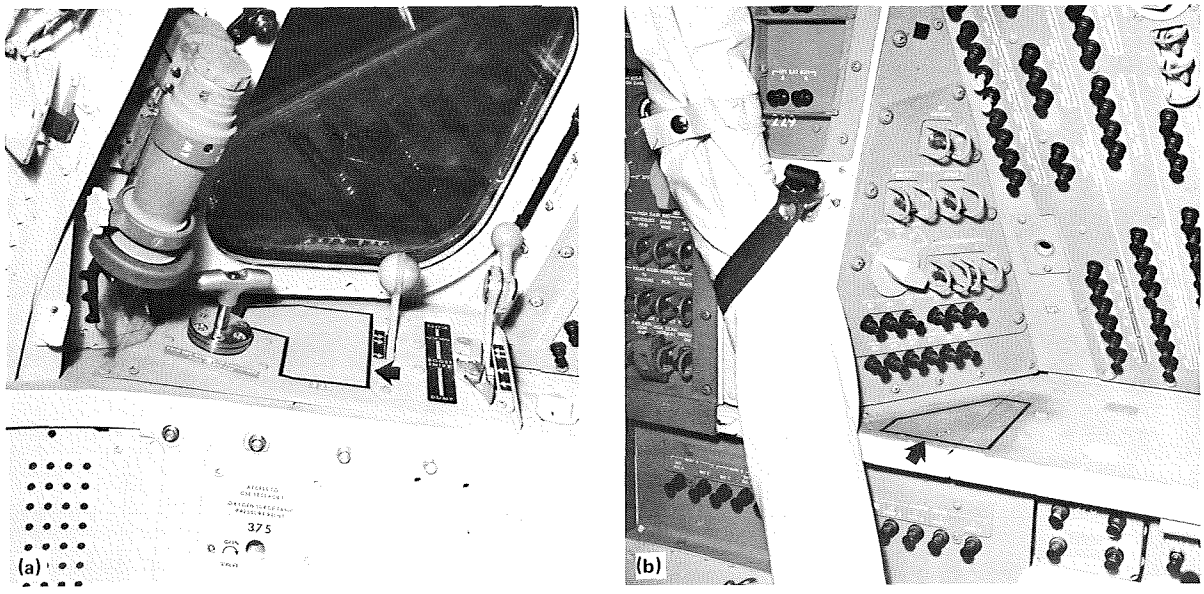

FIGURE 15-3.—Demarcation lines on internal surfaces of Apollo and Soyuz spacecraft. (a) Panel 325 below window in Apollo (S74-33254). (b) Right girth shelf above panel 278 in Apollo (S74-33259). (c) Demarcation lines in Soyuz (AST5-311).

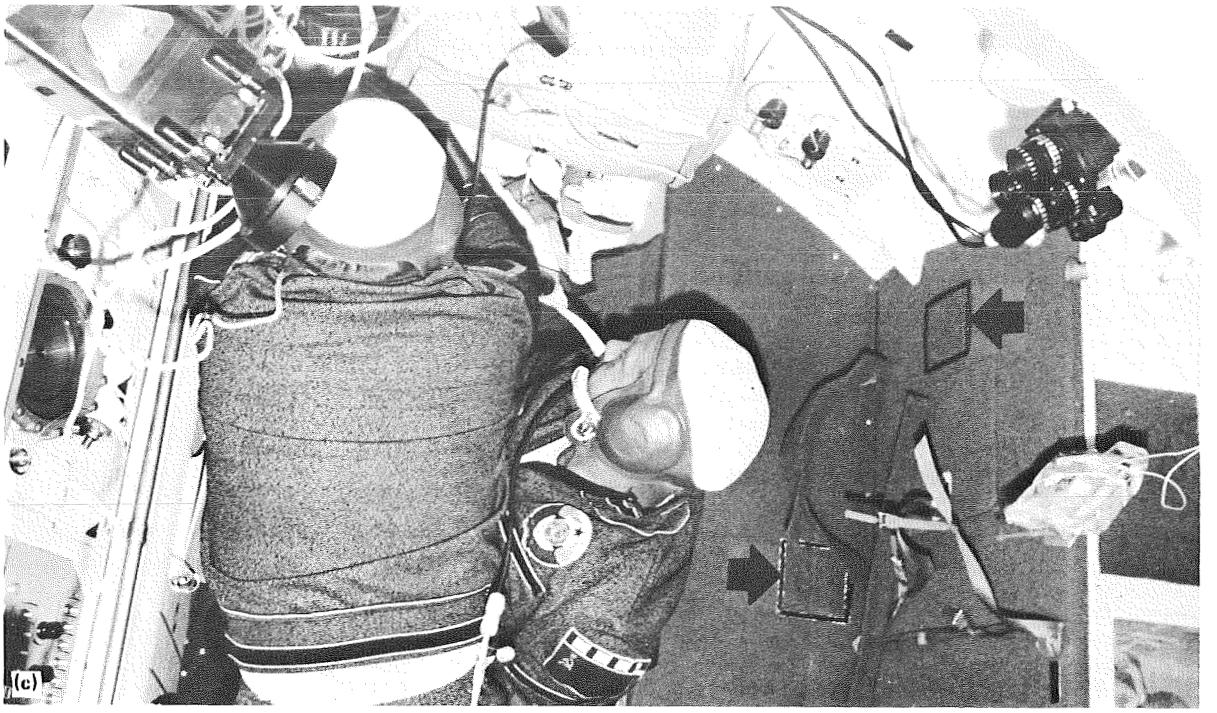

FIGURE 15-3.—Concluded.

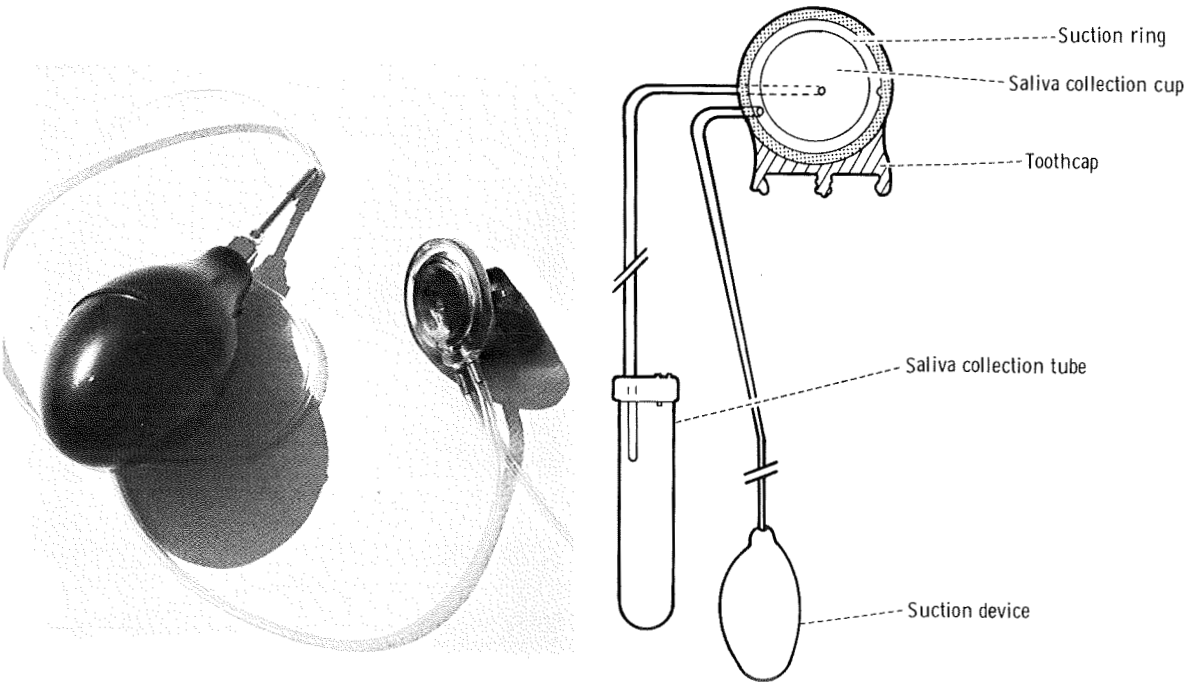

FIGURE 15-4.—Saliva collection device used to obtain salivary parotid fluid.

Preliminary Analysis of Microbial Specimens and Distribution of Test Materials

The contents of each swab and gargle sample were serially diluted under aseptic conditions and subsequently inoculated onto the surface of the nutrient media as previously outlined (ref. 15-16). The variety of media, number of plates inoculated, dilution ranges, and incubation conditions were selected on the basis of what was required to isolate and quantitate the autoflora components present in each sampled area. A common methodology, composed of analytical procedures acceptable to both the U.S. and U.S.S.R. investigators, was used to ensure comparability of data (ref. 15-17). A general schematic summary of the preliminary steps required for specimen distribution is presented in figure 15-5. Following incubation under the appropriate conditions, all resulting colonies on every culture plate were categorized and counted. Subsequently, one sample of each morphologically different colony type was transferred from each dilution series to the appropriate nutrient media and appropriately stained. Following verification that a pure culture was obtained, one aliquot of each culture was stored by the laboratory in which it was identified (the host laboratory), and another complementary aliquot was made available to the guest laboratory. The guest laboratory was to have had access to an isolate from each different type of colony grown on the incubated plates.

Division of Responsibility for Specimen Analysis

The analytical procedures required for performance of the Microbial Exchange Experiment were divided between the laboratories in the United States and the U.S.S.R. (table 15-IV). The U.S. Principal Investigator was responsible for identification of all filamentous fungi, nonlactose-fermenting Gram-negative rods, and members of the genus *Haemophilus*. The U.S.S.R. Principal Investigator was responsible for the identification of yeasts and yeastlike fungi, Gram-positive catalase-negative cocci, Gram-negative cocci, and lactose-fermenting Gram-negative rods. The responsibility for identification of Gram-positive

TABLE 15-IV.—Division of Responsibility for Specimen Analysis

Activity	*Responsible laboratory*	
	U.S.	*U.S.S.R.*
Collection of specimens from astronauts and Apollo spacecraft	X	
Collection of specimens from cosmonauts and Soyuz spacecraft		X
Identification of filamentous fungi	X	
Identification of yeasts and yeastlike fungi		X
Identification of Gram-positive catalase-positive cocci	X	X
Identification of Gram-positive catalase-negative cocci		X
Identification of Gram-negative cocci		X
Identification of nonlactose-fermenting Gram-negative rods	X	
Identification of lactose-fermenting Gram-negative rods		X
Identification of *Haemophilus sp.*	X	
Bacteriophage typing of *Staphylococcus aureus*	X	
Antibiotic sensitivity testing of *Staphylococcus aureus*		X
Determination of immunoglobulin levels in parotid fluid	X	
Determination of steroid, urea nitrogen, uric acid, calcium, and potassium levels in parotid fluid	X	
Determination of lysozyme levels in parotid fluid		X
Determination of bactericidal activity of serum		X
Measurement of viral antibody titers in serum		X
Measurement of specific microbial antibody levels in serum	X	
Determination of leukocyte differential count	X	

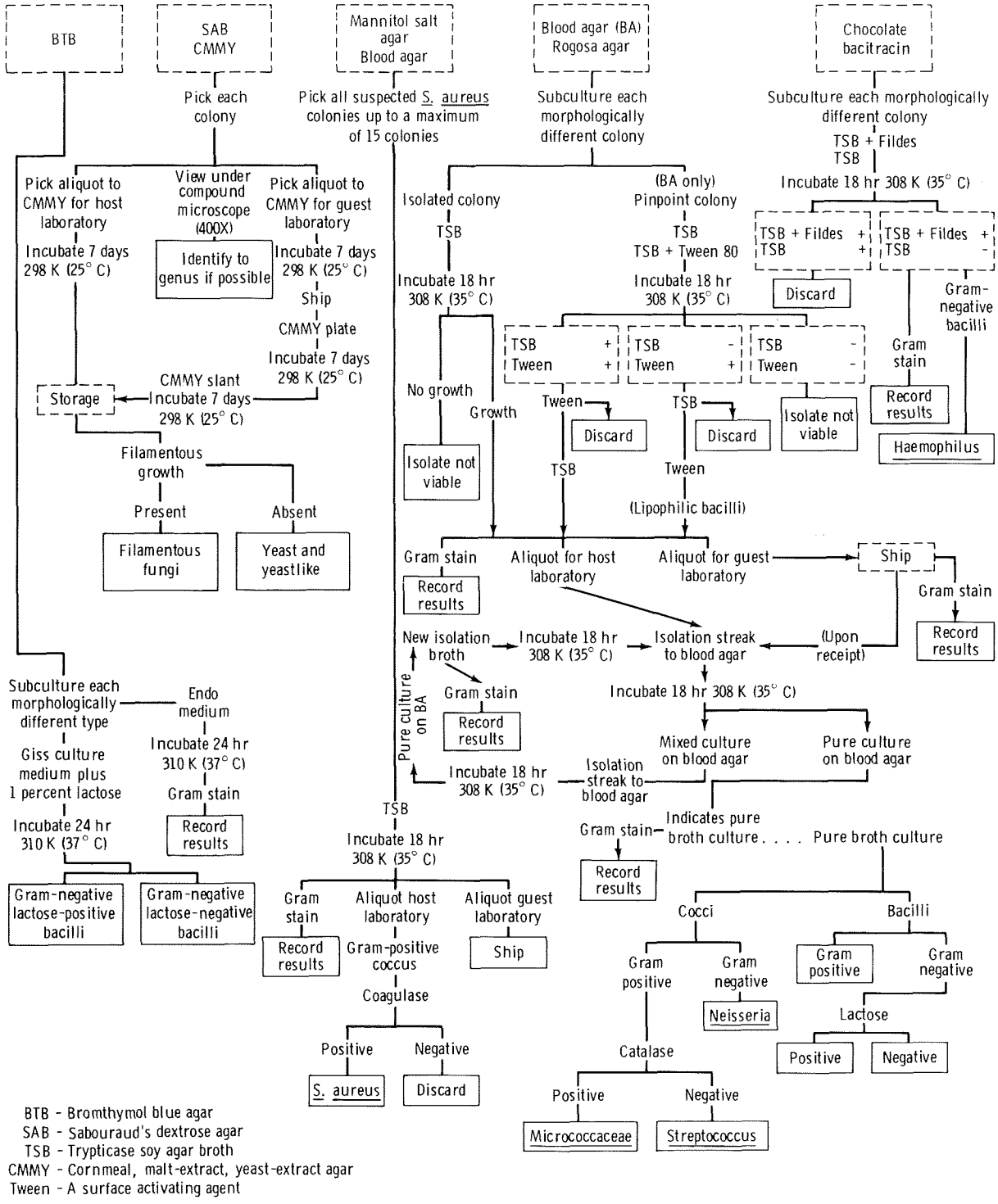

FIGURE 15-5.—Schematic diagram for identifying microorganisms.

catalase-positive cocci was shared by both laboratories. In addition to these microbial identifications, the responsibility for specific tests was divided as shown in table 15-IV.

The proper collection of preflight and postflight samples from the cosmonauts and the Soyuz spacecraft was the responsibility of the U.S.S.R. Principal Investigator. The proper collection of preflight and postflight samples from the astronauts and the Apollo spacecraft was the responsibility of the U.S. Principal Investigator. The safe and expeditious return of samples collected during flight was the responsibility of the U.S.S.R. Principal Investigator. The preliminary analysis of all samples, culminating in and including distribution of specimens to both U.S. and U.S.S.R. investigators, was the responsibility of the Principal Investigator of the country in which the sample was collected. All preliminary specimen analyses conducted in the U.S.S.R. laboratories were to be conducted in the presence of a science representative of both the United States and the U.S.S.R. Although this arrangement was also available in the U.S. laboratories, the U.S.S.R. investigators had the option of exercising this procedure. These joint activities were to include dilution and plating of specimen materials, isolation of microbial colonies, and evaluation of growth properties on the initial isolation media. Crewmembers and spacecraft were sampled independently.

RESULTS AND DISCUSSION

Analysis and Evaluation of Operational Activities

The extent to which each objective of the experiment was accomplished is evaluated together with a discussion of any reported anomalies.

1. The normal microbial autoflora level and the immunocompetence level of each crewmember were identified. The collection of five sets of preflight saliva and microbial samples and of two sets of serum samples for each of the prime and backup crewmembers provided adequate establishment of baselines for U.S. crewmembers. However, data pertaining to the total microbial load could not be retrieved from microbiology specimens collected in the U.S.S.R. All serum samples, with the exception of those taken from the backup SCDR 15 days before launch, were received in a condition that permitted the required analysis. All the astronaut saliva samples, with the exception of the CMP samples, were collected as planned. (An alternate collection method was used for the CMP that provided an adequate but not optimum saliva sample.) All cosmonaut saliva samples were collected in a manner different from that agreed upon and therefore were not usable in this study.

2. The identification of qualitative and quantitative changes in crew and spacecraft microbial populations and the evaluation of demonstrable intercrew microbial exchange occurring during flight were necessary for proper analysis. The most important marker microorganisms were properly monitored in the U.S. and U.S.S.R. laboratories, allowing for a reasonably thorough evaluation of intercrew microbial exchange. Likewise, shifts in many of the major components of the autoflora could be evaluated. Variations in the total autoflora could be calculated only for the Apollo crew.

3. Selected microorganisms were to be critically evaluated to detect postflight changes in their ability to be pathogenic, infective, or toxic in man. Several of the microorganisms that are most useful for these evaluations were repeatedly isolated from Apollo-Soyuz crewmembers. Although these are protracted studies and have not yet been completed, the prognosis for satisfying this objective is very good. Thirty-day postflight samples of cosmonauts were not collected.

4. Immunological parameters of blood and saliva were to be evaluated to detect postflight changes in the ability of crewmembers to resist infection. The required astronaut specimens were obtained and analyzed as planned. Data from cosmonaut specimens were less complete because of incomplete blood collection and different saliva collection techniques.

Microbiological Analysis of Apollo-Soyuz Test Project (ASTP) Crewmembers

On early Apollo missions, before strict protective measures were instituted, in-flight infections were not unusual (ref. 15-18). In-flight illness of microbial origin was, however, completely absent from the Apollo 14 through 17 missions (ref. 15-19). There is no doubt that the implementation of extensive preventive measures (ref. 15-20), following the clinically significant Apollo 13 mission, was a contributing factor. Preflight monitoring of pathogenic and potentially pathogenic species identified certain potential problems so that appropriate prophylaxis or treatment could be administered before the flight or could be provided during the flight. This procedure was highly effective and was recommended for all future U.S. manned space flights (ref. 15-19). Similar analyses conducted during the three Skylab missions indicated that, whereas there were several in-flight disease events and gross contamination of the orbital workshop did occur, such events did not prove to be limiting hazards for long-term space flight (ref. 15-10). The ASTP mission, a unique space flight in which two teams of crewmembers from different geographical areas joined in space with two different spacecraft, presented an unusual opportunity for cross-contamination. Accordingly, it was necessary to identify and trace all aerobic bacterial species as well as all microorganisms of potential medical importance present in the population.

Total microbial load.—One method of detecting important autoflora changes is to monitor alterations in the size of the bacterial population inhabiting the body of the test subject. Accordingly, the number of viable bacterial cells recovered from the skin sites of all five prime crewmembers was determined and is illustrated in figure 15-6(a). These data show no significant differences between subjects, indicating that Apollo and Soyuz crewmembers retained approximately the same microbial load on their skin despite the geographical and cultural differences involved. The data in figure 15-6(a) also show that the values obtained immediately after space flight were within the preflight range, demonstrating that the population

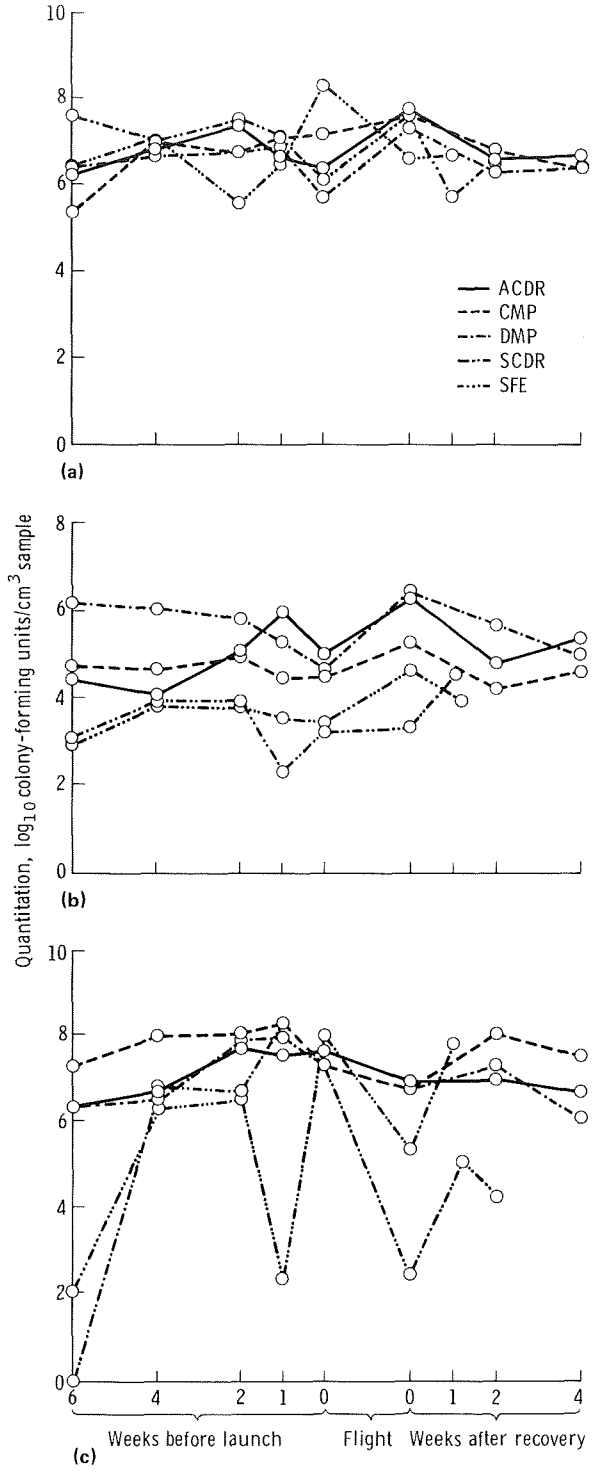

FIGURE 15-6.—Number of aerobic bacterial cells recovered from crewmembers. (a) Cells in skin samples. (b) Cells in nasal cavity. (c) Cells in oral cavity.

of aerobic bacteria (measured in terms of total viable cells) inhabiting the integumentary surfaces was stable following space flight, and that no medically important alterations occurred.

Analyses of the bacterial load recovered from the nasal and oral cavities of ASTP crewmembers were likewise determined and are presented in figures 15-6(b) and 15-6(c), respectively. The upper respiratory tract samples obtained from the cosmonauts yielded counts that were generally lower and more variable than those obtained from astronaut specimens. Even with this variability, the resulting data show that the space flight had no demonstrable effect on the total bacterial load recovered from the oral and nasal cavities.

Contamination of spacecraft internal surfaces.—Multiple sites within the Apollo and the Soyuz spacecraft were sampled before, during, and after the flight. Subsequent analyses resulted in the data illustrated in figure 15-7. Most sampled areas were not sterile before flight and thus afforded a potential source of contamination to the crews. Contamination within the Soyuz spacecraft was increased (by one order of magnitude) the day of return from space flight, a response similar to those previously reported for other space flights (refs. 15-7, 15-17, and 15-21). Contamination within the Apollo spacecraft was measurably decreased after the flight. This situation was probably the result of prolonged contact of the sampled surfaces to a dinitrogen tetroxide and nitrous oxide gas mixture that had inadvertently entered the spacecraft during the landing procedure. It is important to note that this toxic gas affected the contamination level of spacecraft surfaces but did not measurably influence the autoflora of the crewmembers. This occurred because the autoflora components were better protected from outside affectors and had a significantly shorter contact time with the toxic gas.

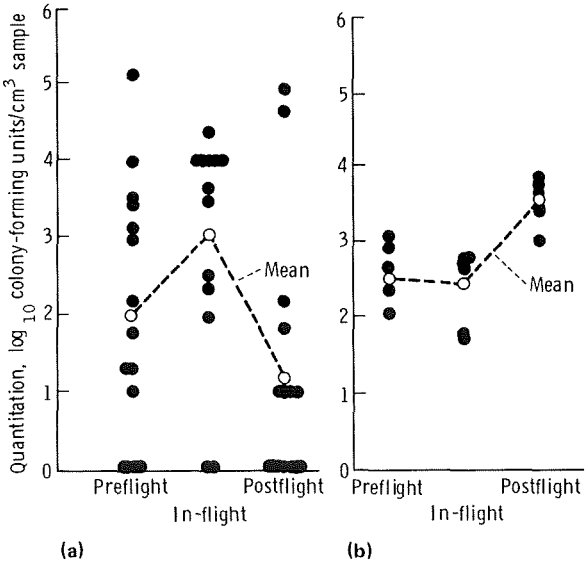

FIGURE 15-7.—Number of aerobic bacterial cells on spacecraft internal surfaces. (a) Apollo. (b) Soyuz.

Gram-negative rods.—A number of different microbes that occur normally in the intestinal tract, or are associated with intestinal infection, are placed in the enteric group of microorganisms. The normally occurring members of this group are generally considered to be of potential medical importance when recovered repeatedly, or in large numbers, from sites other than the lower digestive tract. A complete analysis of the specific Gram-negative rods recovered from Apollo and Soyuz crewmembers has previously been presented (ref. 15-16). Accordingly, these data are only summarized in this report.

It has previously been reported (refs. 15-2 and 15-22) that the Gram-negative rod component of the autoflora could change during or following confinement in a closed environment and that this change could result in "dysbacteriosis," such as a flooding of the oral cavity with a particular species. The data presented in figure 15-8 show that, when considered as a unit, the quantitation of Gram-negative rods is too variable to establish any flight-affected alteration. This is to be expected because these species are mostly transient to the human body rather than being true members of the autoflora (except in the lower gastrointestinal tract, which was not evaluated in this study). There was, however, an increase in the number (incidence) of cosmonaut body sites from which Gram-negative rods were recovered upon return from the space flight. This spreading of bacteria to previously uncontaminated sites probably resulted from the altered hygiene regimen used onboard the Soyuz spacecraft.

Of the Gram-negative rods recovered, only members of the genus *Haemophilus* are indigenous

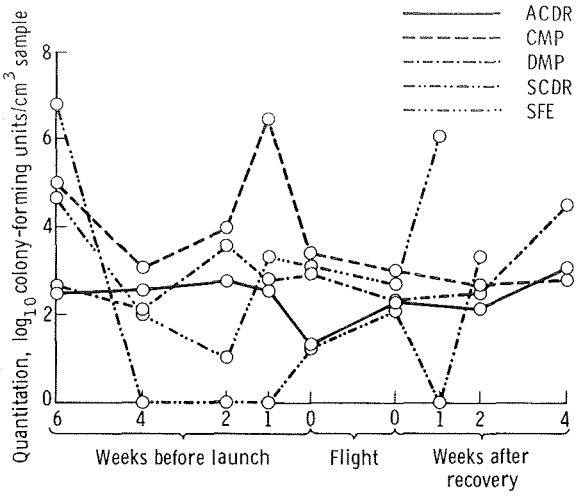

FIGURE 15-8.—Number of Gram-negative rods recovered from crewmembers. (The genus *Haemophilus* is excluded.)

to the human autoflora, being continually present in the oral cavity. Therefore, it is possible to evaluate changes within a single genus of Gram-negative rods only with these microorganisms. The data illustrated in figure 15-9 show that there was a quantitative decrease in the recovery of *Haemophilus* in both prime and backup crewmembers immediately preceding the flight. This

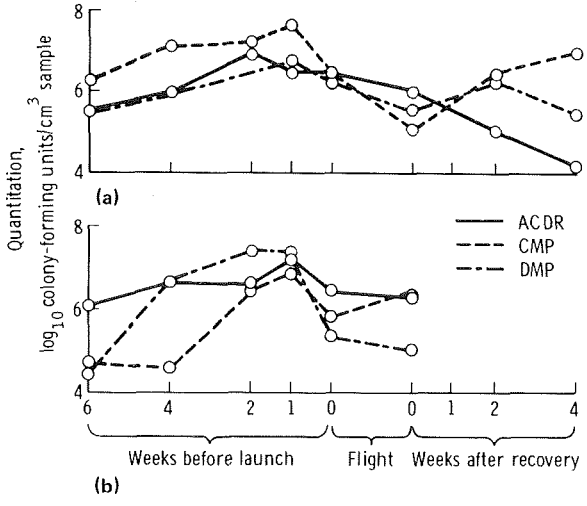

FIGURE 15-9.—Number of *Haemophilus* cells in oral cavity samples from Apollo crewmembers. (a) Prime crew. (b) Backup crew.

decrease coincides with the preflight maintenance of these subjects in protective isolation, a procedure that has been shown to minimize the probability of preflight and in-flight infections in previous space flights (refs. 15-19 and 15-23). The load of *Haemophilus* cells in the oral cavity of all three of the ASTP prime crewmembers was significantly reduced during the flight but remained constant in the nonflight control group. This demonstrates a decrease in the relative importance of these potential pathogens to the autoflora during space flight.

None of the observations previously discussed support the view that Gram-negative rods undergo unfavorable dysbacteriotic changes following space flight. Therefore, those few instances in which such changes have been reported should be considered to be coincidental with, and not a function of, space flight.

Candida albicans.—This species, which has long been recognized as a normal component of the indigenous autoflora of man, has frequently been recovered from the crewmembers of previous space flights and space simulation missions (refs. 15-8, 15-19, 15-24, and 15-25). Because *C. albicans* has been identified as the causative agent for serious oral cavity disease, its presence in the mouth of ASTP crewmembers was carefully monitored. Analysis of the data presented in reference 15-16 indicates an intracrew transfer of this species to the Apollo commander during flight. No such transfer occurred with the Apollo backup controls, although *C. albicans* was routinely carried in the mouth of the backup Apollo CMP. Among the Soyuz cosmonaut microbial population, *C. albicans* was recovered only from the prime FE 33 and 15 days before launch, and therefore was not considered of medical importance during the flight.

Staphylococcus aureus.—Although *S. aureus* microorganisms are not uncommon skin and nasal contaminants, all strains are potential pathogens. The results of several space-flight-simulation studies have suggested increases in the toxigenic activity, virulence, or pathogenicity of this species with stressful confinement of the human host (refs. 15-2, 15-22, and 15-26). The strains of *S. aureus* recovered from the 10 ASTP crewmembers are presented in table 15-V. A more

TABLE 15-V.—Staphylococcus Aureus Strains Carried by Crewmembers

Flight status	*Position*	*Quantitation (a)*		*Predominant bacteriophage type carried*
		Preflight mean	*Postflight*	
		Apollo		
Prime	ACDR	3.04	1.95	Not typeable
	CMP	2.06	0	53, 83A, 85
	DMP	1.10	1.30	52, 52A, 80, 81
Backup	ACDR	1.65	0	3A, 3C
	CMP	3.80	3.86	55, 71
	DMP	3.23	4.83	29, 53, 54, 75, 79, 80
		Soyuz		
Prime	SCDR	2.82	3.36	53, 54, 80, 81
	SFE	3.15	4.65	6, 42D, 42E, 47, 53, 81
Backup	SCDR	2.27	(b)	6, 53, 75, 85
	SFE	2.48	(b)	52, 53

[a] Quantitations given as $\log_{10}$ colony-forming units/cm^3 sample.
[b] No sample collected.

complete analysis is presented in reference 15-16. These data show that each crewmember carried a different strain, each of which is expressed as a numbered bacteriophage type. Type 52, 52A, 80, 81 was carried by the Apollo prime DMP and was transferred to the ACDR during flight. There was no transfer demonstrated between crews.

A postflight increase in the incidence of *S. aureus* has previously been reported (ref. 15-19). This could not occur during the ASTP mission because of the universal presence of this species before flight. However, there was a measurable increase in the number of *S. aureus* cells recovered from prime Soyuz crewmembers immediately after the flight. Although recovery incidence and quantitation of this medically important species was unusually high, no disease events were reported.

Total load of potential pathogens.—Several authors have warned that returning space travelers may experience a "microbial shock" and may respond negatively to renewed contact with potentially pathogenic microorganisms that were absent in the space-flight environment (refs. 15-12, 15-14, and 15-18). These warnings were based on the assumption that contact with potential pathogens during space flight would be very limited, resulting in a reduction of immunocompetence. However, there was no demonstrable decrease in the incidence of medically important microorganisms recovered from the ASTP crewmembers on recovery day, a finding that supports earlier reported results (refs. 15-7, 15-19, and 15-24). Accordingly, no postflight disease events were reported. Therefore, neither the proposed simplification of the population of medically important microorganisms, nor the theorized postflight microbial shock, could be supported by the results of this study.

Evaluation of Immunological Parameters

The effect of space flight on man's immune system has been the subject of much speculation. Ginsberg (ref. 15-27) has reviewed the mechanisms of natural and acquired immunity and has concluded that the factors which contribute to our

natural, nonspecific immunity could be the most impaired during space travel. Nefedov et al. (ref. 15-2) have reported experiences with human subjects at the Soviet Arctic and Antarctic Institutes. Upon contact with healthy replacements, mass disease erupted among subjects who had resided for months in isolation during which only sterile food was consumed. Additionally, studies of animal resistance to infection performed in space-flight simulators have shown that these conditions may reduce the ability of test subjects to resist infectious challenge by viruses (ref. 15-28) and by staphylococci (ref. 15-29) and may cause a reduction of antibody titer and spleen weight when given an antigenic challenge (ref. 15-30). Hypokinesis and acceleration studies have been reported to produce impairment of properdin, neutrophil phagocytosis, salivary lysozyme, skin bactericidal activity, and increased incidence of inflammatory diseases (ref. 15-31).

A variety of humoral immunological responses were noted for three subjects after being confined in a Skylab simulator for 56 days (ref. 15-32). The most significant of these was a rise in the serum immunoglobulins IgG and IgM. Likewise, salivary muramidase increased before entry into the chamber, again at a point midway in the confinement, and once more at the conclusion of the chamber study. During Apollo missions 7 through 11, increases related to illness were observed in IgG, IgM, IgA, haptoglobin, ceruloplasmin, and α_2 macroglobulin (ref. 15-18). Alterations not related to illness were also sometimes observed.

Although many components of the immune mechanism have been studied in connection with U.S. and U.S.S.R. space flights, the response of specific antibodies, produced in response to microorganisms carried by one or more crewmembers, has not yet been evaluated. Accordingly, such a study was incorporated into the Microbial Exchange Experiment and the data were correlated with the appropriate microbial population as well as with salivary IgA and lysozyme levels.

Analysis of parotid saliva.—Automatic, individualized parotid fluid collectors (fig. 15-4) were fabricated to fit the biting surfaces of the molar teeth so that each individual could collect his own parotid secretion with no difficulty. These devices were prepared by the United States for each of the 10 ASTP crewmembers. For reasons that are not readily apparent, the collection devices were not properly used by the U.S.S.R. cosmonauts and parotid fluid of sufficient quantity and quality for detailed analyses was not received for study. Among the U.S. crewmembers, the collecting device could not be used on the prime CMP because of an anomaly in the opening of the parotid duct. Accordingly, data are included only for the remaining two prime and three backup crewmembers.

Laboratory analyses were directed primarily toward establishing rates of flow and studying concentrations of secretory IgA and lysozyme as they might be affected by the mission experience. A summary of the resulting data is shown in table 15-VI. The readings from samples collected from prime crewmembers the day of return from space flight are generally lower than the preflight mean. However, because similar variations occurred in the control (backup crew) population, none of these changes can be shown to be space-flight related. Therefore, there is no reason to suspect that changes in the oral autoflora during space flight are initiated (or influenced) by changes in the IgA or lysozyme content of parotid fluid.

Analyses were continued with samples collected 7, 15, and 30 days after landing of the Apollo spacecraft. Analyses of these data also failed to show any significant ($p \leq 0.05$) alteration in the measured parameters, with the exception of an increase in IgA in the prime DMP sample collected on day R+15. Following exposure to a toxic mixture of dinitrogen tetroxide and nitrous oxide during landing, the crewmembers were treated with medications potentially capable of effecting a reduction in some measured parameters. It is important to note that, notwithstanding this treatment, no significant decreases were observed.

Analysis of blood.—The serum from the blood of U.S. and U.S.S.R. crewmembers was reacted against antigenic preparations of the bacteria *Staphylococcus aureus*, *Streptococcus sp.*, *Haemophilus influenzae*, *Escherichia coli*, and the yeast *Candida albicans*. All of these species were isolated from the crew autoflora. In addition, preparations of *Salmonella typhi* "O" antigen and

TABLE 15-VI.—Analysis of Parotid Saliva From Apollo Crewmembers

Parameter	*Subject*	*Preflight mean*	*Postflight reading and t-test values for comparison to preflight mean*							
			R+0		*R+7*		*R+15*		*R+30*	
			Reading	*t*	*Reading*	*t*	*Reading*	*t*	*Reading*	*t*
Flow rate, cm^3/min	Prime ACDR	0.414	0.16	−1.30	0.49	0.39	0.18	−1.20	0.56	−0.75
	Prime DMP	.530	.43	−.51	.39	−.72	.35	−.91	.38	−.77
	Backup ACDR	.702	.85	.76	--	--	--	--	--	--
	Backup CMP	.386	.37	−.08	--	--	--	--	--	--
	Backup DMP	.390	.54	.77	--	--	--	--	--	--
IgA content, mg %	Prime ACDR	5.36	3.3	−1.85	3.5	−1.67	5.4	0.04	5.4	0.04
	Prime DMP	2.96	.8	−1.94	1.4	−1.40	6.7	3.36	5.2	2.01
	Backup ACDR	3.90	2.5	−1.26	--	--	--	--	--	--
	Backup CMP	5.34	5.1	−.22	--	--	--	--	--	--
	Backup DMP	6.88	2.9	−3.57	--	--	--	--	--	--
Lysozyme content, mg %	Prime ACDR	0.514	0.06	−2.01	0.08	−1.92	0.40	−0.50	0.98	2.06
	Prime DMP	.378	.38	.01	.22	−.70	.68	1.34	.59	.94
	Backup ACDR	1.112	1.10	−.05	--	--	--	--	--	--
	Backup CMP	1.058	.97	−.39	--	--	--	--	--	--
	Backup DMP	1.264	1.43	.74	--	--	--	--	--	--

TABLE 15-VII.—Titers of Species-Specific Antibodies in Serum From Prime Crewmembers[a]

Species	*Sampling time*	*Prime crewmember*				
		ACDR	*DMP*	*CMP*	*SCDR*	*SFE*
Staphylococcus aureus	F−30	64	8	8	8	0
	F−15	64	8	0	8	0
	R+0	8	8	0	8	0
Streptococcus sp. (beta haemolytic)	F−30	16	16	16	32	16
	F−15	16	16	16	32	16
	R+0	16	16	16	32	16
Haemophilus influenzae	F−30	1024	1024	256	64	256
	F−15	1024	1024	256	64	256
	R+0	1024	1024	128	64	128
Escherichia coli	F−30	8	8	0	2048	>8192
	F−15	8	8	0	1024	>8192
	R+0	8	8	0	1024	1024
Salmonella typhi ("O" antigen)	F−30	64	32	64	64	16
	F−15	32	32	64	64	16
	R+0	32	32	64	128	16
Bacillus abortus	F−30	0	0	>8192	>8192	0
	F−15	0	0	>8192	4096	0
	R+0	0	0	>8192	4096	0
Candida albicans	F−30	32	8	16	8	16
	F−15	32	8	8	8	32
	R+0	32	8	0	8	16

[a] Values given are reciprocal of titer. Zero denotes titer of less than 1/8.

Bacillus abortus antigen were likewise reacted against crew serum. The resulting data, expressed as the reciprocal of the highest serum dilution (titer) sustaining a reaction, are presented in table 15-VII. These data illustrate only two significant postflight changes. With *S. aureus*, the titer in the serum of the ACDR was reduced from 1/64 to 1/8 on the day of return from flight (R+0). Likewise, the titer of anti-*E. coli* antibody in the serum of the SFE was reduced from greater than 1/8192 to 1/1024 postflight. Because these were unusual isolated incidences and because all other titers remained essentially unchanged, it must be concluded that this space flight did not effect a demonstrable change in the presence of species-specific antibody.

The results of lysozyme measurements are shown in table 15-VIII. In addition to the considerable variation among individuals, there was a general trend for lysozyme levels to be elevated the day of return from space flight. However, the magnitude of the change was erratic and could not be correlated with the actual space flight. Therefore, these data alone cannot be used as evidence that blood lysozyme levels are affected by space flight.

TABLE 15-VIII.—Levels of Lysozyme in Blood of Prime Crewmembers

Crewmember	*Lysozyme level, $\mu g/cm^3$*		
	F−30	*F−15*	*R+0*
ACDR	13.0	13.0	14.5
DMP	10.0	10.0	10.0
CMP	15.0	15.0	15.2
SCDR	12.5	12.5	12.5
SFE	7.4	7.4	10.0

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